



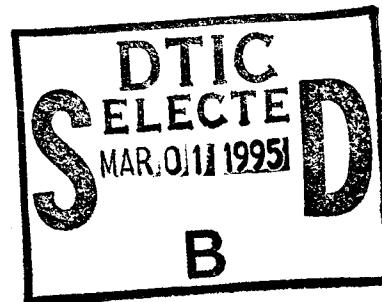
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Zebra Mussel Research Program

Effects of Starvation at Different Temperatures on Dry Tissue and Dry Shell Weights in the Zebra Mussel, *Dreissena polymorpha* (Pallas)

by *R. Chase, Robert F. McMahon,*
Center for Biological Macrofouling Research



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by R. Chase, Robert F. McMahon

Center for Biological Macrofouling Research
Department of Biology
University of Texas at Arlington
P.O. Box 19498
Arlington, TX 76019

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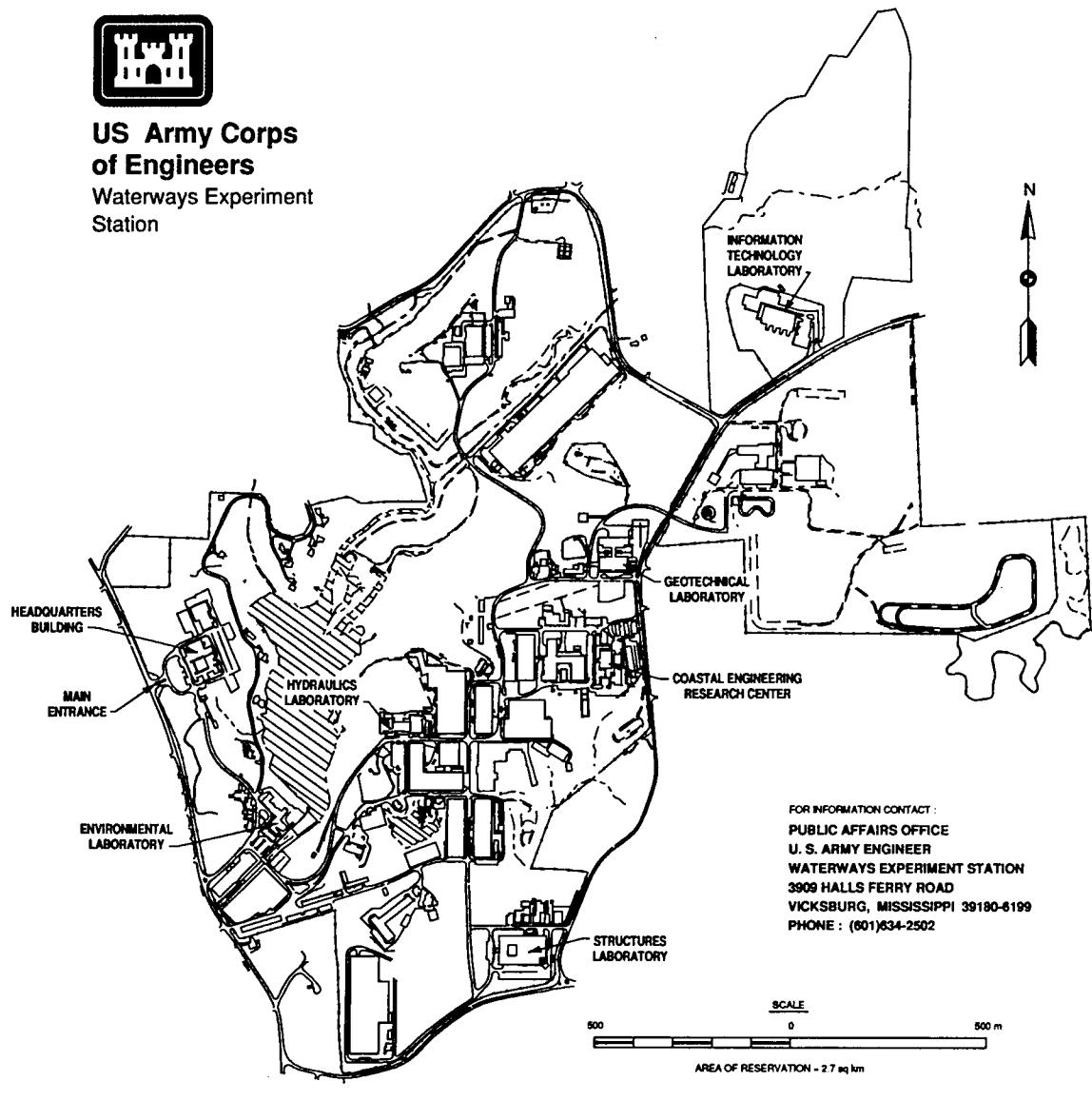
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Preface

The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 specified that the Assistant Secretary of the Army, Civil Works, will develop a program of research and technology development for the environmentally sound control of zebra mussels (*Dreissena polymorpha*). As a result, the U.S. Army Engineer Waterways Experiment Station (WES) initiated a program to develop control strategies for this species.

This report was prepared by Mr. R. Chase and Dr. Robert F. McMahon, Center for Biological Macrofouling Research, University of Texas at Arlington, Arlington, TX. Dr. Milton Matthews and Messrs. Michael Clarke and Thomas Ussery of the Center for Biological Macrofouling Research provided technical assistance during the course of the experiments. Mr. Gary L. Dye, Lockmaster of Black Rock Lock, Buffalo, NY, collected and shipped the zebra mussels utilized in this experiment. This study was supported by a grant to R. Chase from the Ronald E. McNair Post-Baccalaureate Achievement Program, University of Texas at Arlington, and additionally funded under Contract DACW39-92-K-0004 with WES. Drs. Andrew C. Miller and Barry S. Payne, Environmental Laboratory (EL), WES, managed the contract for WES. Dr. Edwin A. Theriot, EL, was Program Manager for the Zebra Mussel Research Program.

During the conduct of this study, Dr. Theriot was Chief, Aquatic Ecology Branch; Dr. Conrad J. Kirby was Chief, Ecological Research Division; and Dr. John W. Keeley was Director, WES.

Dr. Robert W. Whalin was Director of WES at time of publication of this report. COL Bruce K. Howard, EN, was Commander.

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1 Introduction

The “zebra mussel,” *Dreissena polymorpha*, has been a major macrofouling aquatic pest bivalve in Europe for over 200 years. It was introduced to North America in 1986, originally released near the Lake St. Clair-Detroit River region. It is believed that larvae were released with ballast water or that adults escaped anchor chains on ships arriving from Europe (Hebert, Muncaster, and Mackie 1989; Mackie et al. 1989; McMahon, Ussery, and Clarke 1993). Since its introduction, the zebra mussel has spread throughout Lakes Erie, Ontario, Michigan, Oneida, the Finger Lakes, and the Erie-Barge Canal, and the St. Lawrence, Hudson, Oswego, Illinois, Mississippi, Lower Ohio, Lower Tennessee, Lower Arkansas and Lower Cumberland Rivers (Zebra Mussel Information Clearinghouse 1993). Zebra mussels are spreading rapidly throughout U.S. inland waterways through dispersal of adults attached to barge hulls by byssal thread holdfasts and downstream transport of planktonic veliger larvae (McMahon 1992). Isolated populations now extend downstream in the Lower Mississippi River to New Orleans, LA (Zebra Mussel Information Clearinghouse 1993).

Mussels utilize proteinaceous byssal threads to attach to natural hard substrata such as rocks, wood, and macrophytic plants and man-made structures such as metal piping, steel, nylon, fiberglass and wood where they can form encrusting mats many shells thick (4 to 12 in. or 10 to 30 cm) (McMahon 1990). Because of its capacity for macrofouling, the zebra mussel has had major detrimental impacts on recreational boating and commercial shipping as well as on raw water-using industries, potable water treatment plants, and electric power stations which draw from water sources infested with mussels. Presently, the main control technologies for zebra mussel macrofouling center on molluscicides such as chlorine, chlorine dioxide, bromine, ozone, aromatic hydrocarbon compounds, and quaternary ammonium compounds (McMahon 1990). However, federal and state regulations for use of molluscicides are likely to become increasingly restrictive in the future. With nearly every raw water-using facility on the major waterways of the Mississippi Drainage having to apply molluscicides to control zebra mussel fouling, use of molluscicides is likely to become even further restricted in order to prevent water quality degradation and maintain drinking water standards (McMahon, Ussery, and Clarke 1993). Therefore, a high priority has been placed on the development of reliable, cost-effective, environmentally acceptable, nonchemical means of zebra mussel macrofouling control.

Phytoplankton food resources can vary during the year, exposing mussels to prolonged periods of starvation in the winter when algal concentrations are minimal and in the summer when inedible dinoflagellates dominate the plankton or during the algal productivity collapse following the first plankton bloom (Sprung and Borcherding 1991). A search of the available literature reveals that while there have been several investigations of loss of gonadal and digestive tissues during starvation in zebra mussels, there have been few published studies on the effects of prolonged starvation (Sprung and Borcherding 1991, Bielefeld 1991). Loss in wet and dry weight during starvation in the intertidal snail, *Morula granulata* (Duclos), was mainly due to utilization of body organic constituents during extended periods without feeding to provide energy to maintain metabolic functions (Devi, Rao, and Rao 1985). Similar tolerance of extensive loss of body tissue has been reported for the freshwater pulmonate snail, *Planorbella trivolvis* (Russell-Hunter and Eversole 1976). With the exception of that for the freshwater unionid bivalve, *Lamellidens marginalis* (Masthanamma, Purushatham, and Ramamurthi 1984), there have been no studies of overall body tissue weight loss in *D. polymorpha* or any other marine or freshwater bivalve species, nor have temperature effects on starvation rates been investigated. In order to add to the database on starvation effects in zebra mussels and bivalves in general, this study was designed to examine the effects of temperature on whole body dry tissue mass and shell mass in zebra mussels subjected to prolonged starvation over a wide temperature range.

2 Materials and Methods

Specimens of *D. polymorpha* were collected from the guide wall of the U.S. Army Corps of Engineers, Black Rock Navigation Lock on the Niagara River just downstream from Lake Erie, Buffalo, NY. Mussels were flown overnight to Texas emersed on moist paper toweling in cooled insulated containers. On arrival they were maintained at 5 °C in a 284-l refrigerated "Living Stream" holding tank for ≈60 days until utilized in the experiment. As will be detailed in the following chapter, holding mussels at 5 °C prior to experimentation greatly suppresses metabolic rate, making tissue loss negligible over the 60-day pre-experimental holding period.

A sample of mussels was removed from the 5 °C-holding tank on June 15, 1993, and 720 individuals (shell length 15 to 26 mm) were carefully separated from byssally bound mussel clusters using a scalpel to cut threads. Separated individuals were rinsed free of silt and organic debris accumulated on the shells by placing them in a sieve (opening 4.0 mm) and running dechlorinated tap water over them at holding room temperature (15 °C). Three subsamples of 210 animals each were selected at random from the cleaned and separated mussels. They were placed in three 13-l plastic holding tanks that were filled with dechlorinated, City of Arlington tap water. The tanks were held at a constant temperature in either an incubator at 25 °C, a cold room at 15 °C, or a refrigerated water bath at 5 °C. These subsamples were utilized to determine shell and tissue biomass variation over the course of starvation. A further three subsamples of 30 mussels each were placed in 9-cm-diam by 5-cm-high glass crystallization dishes covered with a 1-mm mesh nylon screen to prevent mussel escape. One of these smaller subsamples was placed in each of the 13-l experimental holding tanks and was assessed daily for survivorship. All mussels were maintained without food at the three holding temperatures for the duration of the experiment.

Tanks were inspected daily. Any dead mussels (from either subsample) were immediately removed to prevent contamination. Tanks were held in low light conditions to slow bacterial and algal growth. Water in each tank was aerated and changed three times weekly. Water temperature was taken daily using a standard mercury thermometer ($\pm .1$ °C) and adjusted as needed.

A control sample of 10 mussels was removed from the larger subsample of 210 individuals from each tank on day zero. Initially, subsequent samples of

10 mussels were collected twice a week from mussels held at 25 °C, once a week from those held at 15 °C, and once every two weeks from those held at 5 °C. As the experiment progressed and it became clear that mussels tolerated starvation for much more extended periods than initially predicted, the duration between samples was extended for up to 30 days in order to allow determination of tissue loss throughout the tolerated period of starvation.

A number of parameters were determined for sampled mussels. These included shell length (SL) measured with a dial caliper to the nearest 0.1 mm, and dry tissue weight (DTW) and dry shell weight (DSW) measured to the nearest 0.0001 g. To determine DTW and DSW, sampled specimens were frozen for approximately 24 hr. Freezing allowed tissues to be more completely excised from the shells with a scalpel than could be achieved with wet tissues at room temperatures. Excised tissues were defrosted on paper towels, allowing absorption of extracorporal mantle cavity water. Separated wet tissues and shells were dried to constant weight for 24 to 26 hr at 65 °C.

Mortality was determined daily in the smaller subsamples ($n = 30$) of mussels. The glass crystallization dishes containing these samples were removed from the holding tanks and the nylon screen removed. Mussels stayed submerged in the media remaining within the crystallization dishes while their viability was determined. All mussels widely gaping the valves and unresponsive to strong stimulation of exposed siphon and mantle tissues with the tip of a blunted dissection needle (i.e., did not close the valves) were considered dead and removed from the sample.

3 Results

A 100-percent sample mortality was achieved in mussels at 25 °C after 166 days of starvation. In contrast, no mortality had occurred in mussels starved at either 5 or 15 °C after 229 days of starvation. Estimated by probit analysis (Bliss 1936), the LT_{50} value (estimated time for 50-percent sample mortality) for the 25 °C mussel sample was 118 days and the LT_{100} (estimated time for near 100-percent sample mortality), 143 days.

Multiple Least Squares Linear Regression analysis indicated that 61 percent of the total variation in the natural logarithm (ln) of DTW was explained by correlation with days of starvation, test temperature, and individual shell length. An extremely high correlation of ln DTW with test temperature ($P < 0.00001$) suggested little or no tank effects on DTW during starvation, allowing subsequent statistical analyses to utilize individual mussels as the experimental unit.

Multiple Factor Analysis of Variance was carried out on the mean natural logarithmically transformed values of DTW among temperature treatments with SL and days of starvation as covariants. The analysis indicated a strong positive correlation of ln DTW with SL and strong negative correlation with days of starvation ($P < 0.00001$) (Table 1). Holding temperature significantly affected DTW ($P < 0.00001$). A Student Newman-Keuls Multiple Range Test revealed that mussels held at the three test temperatures all had mean dry tissue weights that were significantly ($P < 0.05$) different from those held in the other temperature treatments with mussels held at 25 °C having the lowest DTW and those held at 5 °C, the highest (Table 1).

A similar Multiple Factor Analysis of Variance revealed no significant effects of holding temperature on the ln DSW of starving mussels ($P = 0.236$) and no correlation with days of starvation ($P = 0.310$) while, as expected, there remained a strong correlation of ln DSW with SL ($F = 648$, $P < 0.00001$).

For each sequential subsample of living mussels, a least squares semi-logarithmic linear regression of ln DTW or ln DSW versus SL was determined. The vast majority of these regressions were significant ($P < 0.05$) for all samples among all test temperature groups. In a few cases, limited size

Table 1

Multiple Factor Analysis of Variance Testing the Significance of Holding Temperature (5, 15, or 25 °C) on the Natural Logarithm of Dry Tissue Weight in Starving Specimens of *Dreissena polymorpha* With Days of Starvation and Shell Length as Covariants

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariant Days Starvation	17.48	1	17.48	107.32	<0.00001 ¹
Covariant Shell Length	90.96	1	90.96	558.38	<0.00001 ¹
Main Effect Holding Temperature	16.43	2	8.23	50.42	<0.00001 ¹
Residual	75.23	462	0.162898		
Total	196.12	466			

¹ Indicates a significant correlation or effect at $P \leq 0.05$.

Student Newman-Keuls Multiple Range Analysis of the Differences Between the Mean Natural Logarithm of Dry Tissue Weights of Specimens of *Dreissena polymorpha* Subjected to Prolonged Starvation at a Test Temperature of 5, 15, or 25 °C

Test Temperature, °C	n	Mean In Dry Tissue Weight, mg	Significant Diff. ¹ (P < 0.05)
5	117	-4.3812	a
15	160	-4.1171	b
25	190	-3.8784	c

¹ Means with the same letter are not significantly different at the 0.05 level.

ranges of samples led to insignificant regressions, $P > 0.05$. The average SL of all individuals in all samples from all three test temperatures was approximately 20 mm. The regression of ln DTW versus SL for individuals sampled on day zero from the three test temperature control groups ($\ln DTW = -6.837 + 0.145 (\text{mm SL})$: $n = 30$, $r = 0.819$, $F = 57.2$, $P = <0.00001$) was used to estimate DTW of a mussel with an average SL of 20 mm as 19.7 mg.

The individual SL versus ln DTW or ln DSW regressions for each sample were then utilized to estimate the DSW and DTW for an average size, standard 20-mm-SL mussel. The estimated DSW or DTW of a standard mussel determined from each sample regression was then fitted to a least squares

linear regression as the dependent variable versus time in days of starvation as the independent variable (Table 2). These regressions revealed that there was no significant shell weight loss ($P < 0.05$) among zebra mussels held at 5 °C after 229 days of starvation or at 25 °C over 132 days of starvation. However, individuals held at 15 °C displayed a significant trend ($P < 0.05$) toward increasing DSW over the 229-day period of starvation (Figure 1, Table 2).

Table 2

Parameters of Least Squares Linear Regression Equations Relating the Estimated Milligrams Dry Tissue Weight (DTW) or Dry Shell Weight (DSW) of a Standard, 20-mm-Long Specimen of *Dreissena polymorpha* (Dependent Variables) to Days of Starvation at Test Temperatures of 5, 15, or 25 °C

Temp., °C	DTW/DSW, g	a (Intercept)	b (Slope)	n	r	F	P
5	DTW	20.69	-0.0207	12	-0.603	5.74	<0.05 ¹
15	DTW	18.79	-0.0386	16	-0.748	17.77	<0.0025 ¹
25	DTW	18.22	-0.1028	19	-0.876	55.96	<0.0001 ¹
5	DSW	319.24	0.0050	12	0.048	0.025	>0.50
15	DSW	316.12	0.1485	16	0.561	6.41	<0.05 ¹
25	DSW	320.98	0.0629	19	0.156	0.423	>0.5

¹ Indicates significant correlation at $P \leq 0.05$.

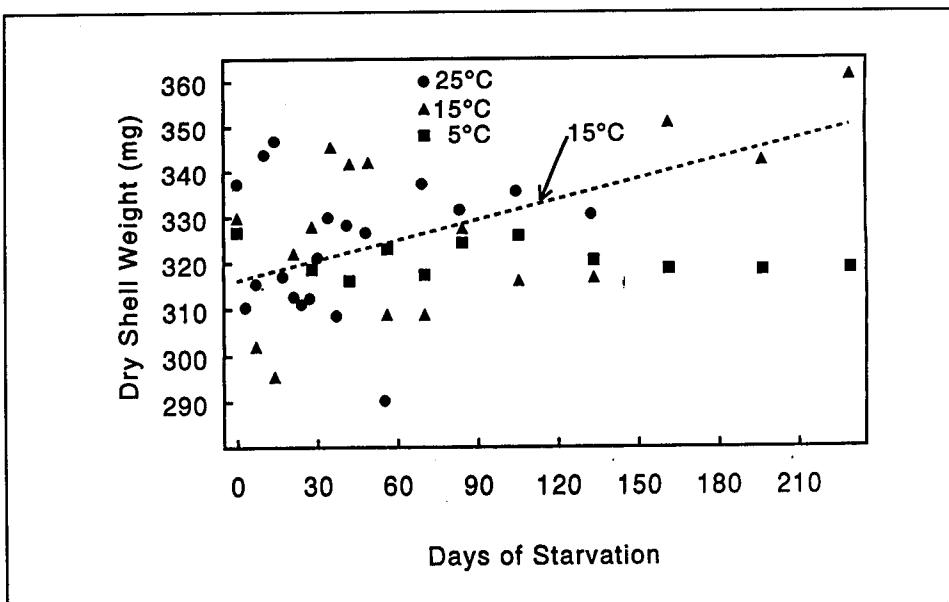


Figure 1. Dry shell weight (DSW in mg, vertical axis) versus days of starvation (horizontal axis) in an average-sized zebra mussel (*Dreissena polymorpha*) (shell length = 20 mm)

In contrast, regressions relating the DTW of a standard 20-mm-SL mussel to days of starvation indicated a significant reduction in dry tissue weight ($P < 0.05$) at all three test temperatures. The slopes of these regressions indicated that mussels starving at 25 °C lost tissue mass 2.7 times faster than did mussels at 15 °C and 5 times faster than did mussels at 5 °C. DTW loss at 15 °C was 1.9 times greater than that of mussels at 5 °C (Figure 2, Table 2).

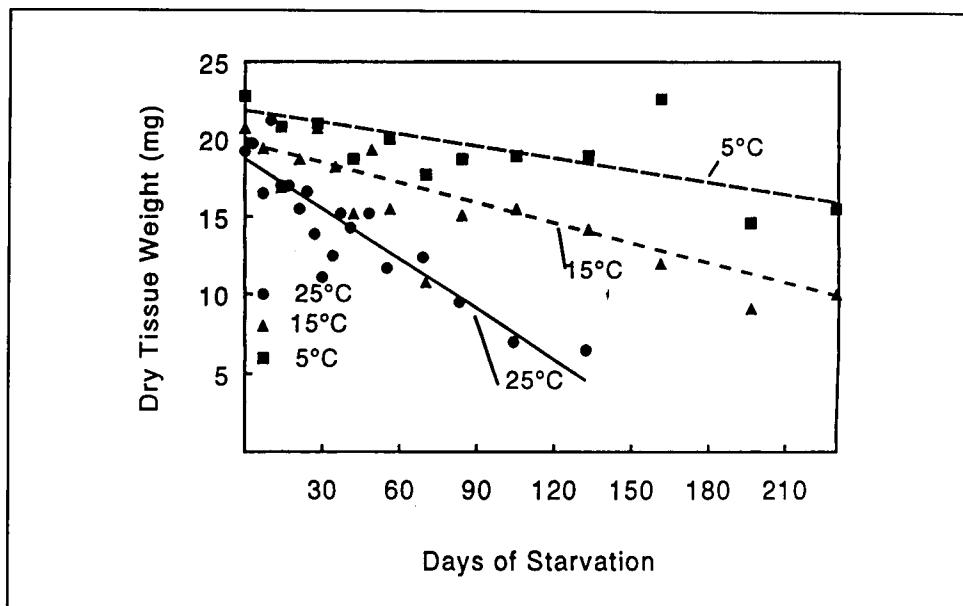


Figure 2. Dry tissue weight (DTW in mg, vertical axis) versus days of starvation (horizontal axis) in an averaged sized zebra mussel (*Dreissena polymorpha*) (shell length = 20 mm)

The regressions of DTW versus days of starvation (Table 2) were utilized to estimate the dry tissue mass lost over the period of starvation. After 132 days, mussels at 25 °C lost 73.8 percent of dry tissue body mass just prior to death. For mussels held 229 days at 5 and 15 °C, dry tissue mass loss was 22.9 percent and 46.9 percent, respectively, and had not reached lethal levels. Based on an estimate that zebra mussel tissue is approximately 50-percent protein and 50-percent carbohydrate, 911 $\mu\text{l O}_2$ would be required for complete aerobic oxidation of one milligram of dry mussel flesh (Hill and Wyse 1989). Utilizing this estimate, the oxygen uptake rate of an average 20-mm-SL, 19.7-mg DTW mussel starving for 132 days at 25 °C was estimated to be $3.86 \mu\text{l O}_2 \cdot \text{animal}^{-1} \cdot \text{h}^{-1}$ or $0.196 \mu\text{l O}_2 \cdot \text{mg DTW}^{-1} \cdot \text{h}^{-1}$ (Table 3). This oxygen uptake rate during starvation was approximately 22.1 percent that of a fed 20-mm-SL mussel at 25 °C (J. E. Alexander and R. F. McMahon, unpublished results). Similar values for zebra mussels starving for 229 days at 15 and 5 °C were estimated to be $1.64 \mu\text{l O}_2 \cdot \text{animal}^{-1} \cdot \text{h}^{-1}$ or $0.0837 \mu\text{l O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$ and $0.786 \mu\text{l O}_2 \cdot \text{animal}^{-1} \cdot \text{h}^{-1}$ or $0.0399 \mu\text{l O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$, respectively, or 13.5 and 8.5 percent of the oxygen

consumption rates of similarly sized fed mussels (Alexander and McMahon, unpublished results) (Table 3).

Table 3 Dry Tissue Weight (DTW) Loss Rates in a Starving, Standard 20-mm Shell Length Specimen of <i>Dreissena polymorpha</i> Converted to Oxygen Consumption Rates by Assuming a Value of 911 $\mu\text{l O}_2$ Required to Aerobically Oxidize 1 mg of Molluscan Dry Tissue						
Temp °C	Days Starved	mg DTW Lost	Vol. O_2 to Oxidize Lost Tissue	$\mu\text{l O}_2$ mg·Day	$\mu\text{l O}_2$ mg·hr	Percent of Nonstarved O_2 Uptake Rate
5	229	4.75	4,327.3	0.959	0.0399	8.5
15	229	9.95	9,064.5	2.009	0.0837	13.5
25	132	13.46	12,262.1	4.715	0.196	22.1

4 Discussion

Zebra mussels appear to be extremely tolerant of starvation, with a mean tolerance time of 125.9 days (S.D. = ± 29.6) at 25 °C corresponding to an LT₅₀ of 118 days and an LT₁₀₀ of 143 days. Mussels held at 5 and 15 °C have survived greater than 229 days without mortality. At 25 °C, mussels tolerated a loss of approximately 75 percent of their dry tissue biomass before dying. Under conditions close to that of overwintering, a 50-percent reduction in tissue biomass of the freshwater snail *Planorbella trivolvis* was observed to occur over 132 days of starvation with only a 10-percent mortality (Russell-Hunter and Eversole 1976). The freshwater bivalve *Corbicula fluminea* and the unidentified dark species of *Corbicula* (*Corbicula* sp.) survived 154 days of starvation at room temperature (22 to 24 °C) under holding conditions similar to those used for zebra mussels (Cleland et al. 1986). Specimens of both species of *Corbicula* tolerated an approximately 75-percent loss of their dry tissue biomass (Cleland and McMahon, unpublished results), data very similar to that reported here for *D. polymorpha*. If approximately 75 percent of dry tissue weight loss is required to induce death in starving zebra mussels, the regression equations relating the decrease in DTW of a standard 20-mm-SL zebra mussel during prolonged starvation (Table 2) can be utilized to estimate that mussels starving at 5 °C will require 750 days to attain a lethal 75-percent tissue loss while those at 15 °C will require 365 days. When this method of estimating lethality was applied to mussels held at 25 °C, the resulting value of 133 days was extremely close to the actual recorded mean time to death of 125.9 days.

The effects of prolonged starvation on shell biomass in *D. polymorpha* were mixed. Shell weight did not change significantly over the entire tolerated 132-day period of starvation in mussels held at 25 °C or in mussels starved at 5 °C for 229 days. In contrast, a small but significant increase in DSW was recorded in mussels starved for 229 days at 15 °C (Table 2, Figure 2). There is a paucity of published results on the effects of starvation on shell biomass in molluscs. A significant loss in dry shell weight was observed after 130 days of starvation in the freshwater unionid mussel, *Lamellidens marginalis* (Masthanamma, Purushotham, and Ramamurthi 1984). In this species, it was suggested that organic (primarily proteinaceous) shell components were being reabsorbed to maintain tissue metabolic demands no longer supported by assimilation of organic nutrients across the gut wall (Masthanamma, Purushotham, Ramamurthi 1984). The increase in shell

biomass in starving mussels at 15 °C may be a result of starvation-induced inhibition of secretion of new shell material at the edge of the shell, thus slowing or preventing increase in shell length while continued mantle secretion of new nacre to the inside of the shell allowed thickening of the shell and a corresponding increase in shell biomass. McMahon and Whitehead (1987) demonstrated a similar increase in shell thickness and biomass in naturally slow-growing populations (due to reduced food availability) of the European freshwater, pulmonate, limpet snail, *Ancylus fluviatilis*. At 25 °C, mussels may not have survived long enough to secrete detectable amounts of new nacre to the inside surface of the shell. At 5 °C, significant amounts of new nacre may not have been secreted by the end of the 229-day starvation period due to the greatly reduced metabolic rate at this temperature (Table 3).

Starving mussels displayed an approximate 75-percent loss of DTW over the tolerated 132-day period of starvation at 25 °C, suggesting that they were extensively metabolizing tissue organic energy stores. Similarly, tissue losses of 22.9-percent and 46.9-percent were recorded in mussels starving at 5 and 15 °C, respectively. The estimated 4.5-, 7.4-, and 11.8-fold reductions in oxygen uptake rates in starving zebra mussels at 25, 15, and 5 °C, respectively (Table 3), suggest that, during starvation, metabolic rates are suppressed to conserve energy stores and prolong the tolerated period of starvation. A similar 4-fold inhibition of O₂ uptake rate has been reported for starving marine blue mussels, *Mytilus edulis* L. (Bayne 1973). However, other mollusc species, including *L. marginalis* (Masthanamma, Purushotham, and Ramamurthi 1984) and the marine gastropod, *Thais lamellosa* (Stickle and Duerr 1970) either maintain a constant metabolic rate at prestarvation levels or display an increased metabolic rate during starvation. Clearly, lack of capacity to suppress metabolic demand during periods of low or no food availability would act to greatly reduce the tolerated period of starvation. The apparent capacity for zebra mussels and blue mussels to greatly suppress metabolic demands when starved thus appears to be an evolved adaption that allows survival of extended periods of low food availability by reducing the rate of organic energy store utilization.

Of interest is the apparent capacity of *D. polymorpha* to sustain increasingly greater reductions in metabolic rate at lower ambient temperatures. Starving mussels held at 5 °C were able to reduce estimated metabolic demand by a 2.6-fold greater extent than could individuals held at 25 °C (Table 3). The extremely low metabolic and tissue biomass loss rates of starving zebra mussels at low temperatures (≤ 5 °C) are almost certainly an adaptation that allows this species to survive long winter periods without feeding while still retaining the vast majority of its cellular organic energy stores for support of reproductive effort as temperatures rise above the spawning threshold in spring. The very reduced metabolic rates and tissue biomass loss rates of mussels starving at 5 °C also strongly suggest that storage of zebra mussels for periods of up to six months at 5 °C in well-aerated water without feeding will have minimal impact on physiological condition when mussels are later used in experimentation at higher temperatures (McMahon, Shipman, and Long 1992).

The apparent ability of zebra mussels to suppress metabolic rate, allowing tolerance of greatly extended periods of starvation even at relatively high ambient water temperatures, makes mitigation of zebra mussel macrofouling by filtration or other means of removal of the mussel's planktonic algal food sources from intake water appear generally impractical. Mitigation by starvation would have to involve utilization of alternative sources of water such as well water or treated, filtered tap water in an infested raw water system for extremely long periods. The algal and bacterial food of zebra mussels is far too small (< 10 μm in diameter) to be efficiently mechanically filtered from intake water. However, if a zebra mussel-infested raw water system such as a fire protection system was switched to an alternative tap or well water source for make-up water, the long period required for mortality by starving mussels might be of some advantage. The prolonged period over which starving mussels would die could prevent sudden downstream fouling of small-diameter components by massive release of dead mussel shells within the system, as could occur with more rapidly acting mitigation technologies (i.e., thermal or molluscicide treatments). Mortality over a long period in such systems could allow relatively easy removal of shells by periodic system flushing.

References

Bayne, B. (1973). "Aspects of the metabolism of *Mytilus edulis* during starvation," *Netherl. J. Sea Res.* 7, 399-410.

Bielefeld, U. (1991). "Histological observation of gonads and digestive gland in starving *Dreissena polymorpha* (Bivalvia)," *Malacologia* 33(1-2), 31-42.

Bliss, C. I. (1936). "The calculation of the time mortality curve," *Ann. Appl. Biol.* 24, 815-852.

Cleland, J. D., McMahon, R. F., and Elick, G. (1986). "Physiological differences between two morphotypes of the Asian clam, *Corbicula*," *Am. Zool.* 26, 103A.

Devi, V. U., Rao, U. P., and Rao, D. G. V. Prasada. (1985). "Body component indices of an intertidal gastropod *Morula granulata* (Duclos) subjected to starvation," *Indian J. Mar. Sci.* 14, 44-45.

Hebert, P. D. N., Muncaster, B. W., and Mackie, G. L. (1989). "Ecological and genetic studies on *Dreissena polymorpha* (Pallas): A new mollusc in the Great Lakes," *Can. J. Fish. Aquat. Sci.* 46, 1,587-1,591.

Hill, R. W., and Wyse, G. A. (1989). *Animal physiology*. 2nd ed., Harper and Row, New York.

Mackie, G. L., Gibbons, W. N., Muncaster, B. W., and Gray, I. M. (1989). *The zebra mussel, Dreissena polymorpha: A synthesis of European experiences and a preview for North America*. Water Resources Branch, Great Lakes Section, Ontario Ministry of the Environment, Toronto, Ontario.

Masthanamma, P., Purushotham, K. R., and Ramamurthi, R. (1984). "Metabolism of *Lamellidens marginalis* (Lamarck) in relation to starvation stress. I. Mortality, body weight, and oxygen consumption," *Indian J. Comp. Anim. Physiol.* 2, 60-63.

McMahon, R. F. (1990). *The zebra mussel: U.S. utility implications*. GS-6995, Research Project 1689-24, Electric Power Research Institute, Palo Alto, CA.

McMahon, R. F. (1991). "Mollusca: Bivalvia." *Ecology and classification of North American freshwater invertebrates*. J. H. Thorp and A. P. Covich, ed., Academic Press, Inc., New York, 315-399.

McMahon, R. F. (1992). "The zebra mussel - the biological basis of its macrofouling and potential for distribution in North America." Reprint # 342 from *Corrosion 92* (Proceedings of the 1992 Annual Meeting of the National Association of Corrosion Engineers), National Association of Corrosion Engineers, Houston, TX.

McMahon, R. F., and Whitehead B. E. (1987). "Environmental induction of shellmorphometric variation in the European stream limpet, *Ancylus fluviatilis* (Müller) (Mollusca: Pulmonata)," *Am. Malacol. Bull.* 5, 105-124.

McMahon, R. F., Shipman, B. N., and Long, D. P. (1992). "Laboratory efficacies of nonoxidizing molluscicides on the zebra mussel (*Dreissena polymorpha*) and the Asian clam (*Corbicula fluminea*)."*Zebra mussels: Biology, impacts and control*. T. F. Nalepa and D. W. Schloesser, ed., Lewis Publishers, Boca Raton, FL, 575-597.

McMahon, R. F., Ussery, T. A., and Clarke, M. (1993). "Use of emersion as a zebra mussel control method," Contract Report EL-93-1, U.S. Army Engineer, Waterways Experiment Station, Vicksburg, MS.

Russell-Hunter, W. D., and Eversole, A. G. (1976). "Evidence for tissue degrowth in starved freshwater pulmonate snails (*Helisoma trivolvis*) from tissue, carbon, and nitrogen analysis," *Comp. Biochem. Physiol.* 54A, 447-453.

Sprung, M., and Borcherding, J. (1991). "Physiological and morphometric changes in *Dreissena polymorpha* (Mollusca: Bivalvia) during a starvation period," *Malacologia* 33(1-2), 179-191.

Stickle, W. B., and Duerr, F. G. (1970). "The effects of starvation on the respiration and major nutrient stores of *Thais lamellosa*," *Comp. Biochem. Physiol.* 33, 689-695.

Zebra Mussel Information Clearing House. (1993). "North American range of the zebra mussel as of 15 December 1993," *Dreissena Polymorpha Information Review* 4(2), 8-9.

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13. ABSTRACT (Maximum 200 words)	<p>Starvation effects on dry tissue and shell biomass were investigated in the zebra mussel, <i>Dreissena polymorpha</i>, at 5, 15, and 25 °C. Subsamples of 30 individuals were examined daily for mortality. A second group was periodically randomly subsampled for dry tissue and shell weights. At 25 °C, 100-percent mortality occurred after 166 days. No mortality occurred at 5 or 15 °C after 229 days. Dry shell weight (DSW) was constant in mussels starved at 25 or 5 °C, but increased significantly ($P < 0.05$) at 15 °C, likely due to deposition of shell nacre in shells not increasing in length. Dry tissue weight (DTW) in starving mussels was significantly correlated with both shell length (SL) and days of starvation ($P < 0.00001$) and was significantly lower at higher test temperatures. Pre-starvation DTW of a 20-mm SL individual was 19.7 mg. DTW declined to 4.66 mg after 132 days at 25 °C, and to 9.95 and 15.9 mg after 229 days at 15 and 5 °C, respectively, corresponding to tissue biomass reductions of 73.8, 46.9, and 22.9 percent. DTW loss rates of 0.102, 0.043, and 0.021 mg/day were recorded for standard mussels starving at 25, 15, and 5 °C, respectively. As estimated from DTW loss rates, O₂ consumption rates in starving standard mussels were 0.196 $\mu\text{l O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$ at 25°C, 0.0837 $\mu\text{l O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$ at 15 °C, and 0.0399 $\mu\text{l O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$ at 5 °C, approximately 22.1, 13.5, and 8.5 percent, respectively, of O₂ uptake rates recorded directly in nonstarved individuals. Mussels held at 25 °C lost 73.8 percent of body mass just prior to death, suggesting that an ≈75-percent DTW loss is</p> <p style="text-align: right;">(Continued)</p>		
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lethal and indicating that 365 days of starvation will be required for 100-percent mortality of mussels at 15 °C and 747 days at 5 °C. Extensive starvation tolerance in *D. polymorpha* is associated with ability to greatly reduce metabolic demand. As this capacity is maximized at low temperatures, mussels may survive winter months with minimal energy store reduction. Extensive tolerance makes starvation impractical for mitigation of *D. polymorpha* fouling.